ELECTRON CAPTURE GAS CHROMATOGRAPHY OF DRUGS. STUDIES ON AMINE DERIVATIVES

PER HARTVIG

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Av

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farm lic

Farmaceutiska fakulteten
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ELECTRON CAPTURE GAS CHROMATOGRAPHY OF DRUGS. STUDIES ON AMINE DERIVATIVES

List of publications

In this survey the following papers will be discussed and referred to by the Roman numerals given in the following list. Some additional preliminary results are also presented.

I. J. Vessman, P. Hartvig and S. Strömberg. Gas chromatography and electron capture detection of benzophenone formed by chromic acid oxidation. Part 3. Applications in the determination of gem-diphenyl substituted compounds. Acta Pharm. Suecica 7, 373 (1971)

II. P. Hartvig. Gas chromatography and electron capture detection of benzophenone. Part 9. Identification of the chromic acid oxidation product of pipradrol. Acta Pharm. Suecica 11, 109 (1974)

III. P. Hartvig, H. Sundin och J. Vessman. Gas chromatography and electron capture detection of benzophenone. Part 7. Permanganate oxidation of gem-diphenyl substituted compounds. Acta Pharm. Suecica 9, 269 (1972)

IV. J. Vessman, P. Hartvig and M. Molander. Electron capture gas chromatography of tertiary amines after a demethylation reaction to secondary amines. Anal. Letters 6 (8), 699 (1973).

V. P. Hartvig and J. Vessman. Electron capture gas chromatography of tertiary amines after a demethylation reaction to secondary amines. Part 2. Studies of different chloroformates and alternative hydrolysis procedures. Acta Pharm. Suecica 11, 115 (1974)

VI. P. Hartvig and J. Vessman. Determination of tertiary amines as pentafluorobenzyl carbamates by electron capture gas chromatography. Anal. Letters. In press (1974)

VII. P. Hartvig, G. Freij and J. Vessman. Electron capture gas chromatography of terodiline after heptafluorobutyrylation. Acta Pharm. Suecica 11, 97 (1974)

VIII. J. Vessman and P. Hartvig. Gas chromatography and electron capture detection of benzophenones. Part 6. Effects of structure and temperature on the sensitivity. Acta Pharm. Suecica 8, 235 (1971)

IX. J. Vessman and P. Hartvig. Gas chromatography and electron capture detection of benzophenones. Part 8. Study of the sensitivity in pulsed and D. C. mode ^{63}Ni high temperature detectors. Acta Pharm. Suecica 9, 463 (1972)

Determination of Drugs by Electron Capture Gas Chromatography

In recent years an increased need for the quantitative determination of drugs and other organic compounds in biological material has arisen. Pharmacokinetic studies in man and animal often require that nano- and picogram amounts of a drug can be analyzed in serum or urine. Metabolic studies as well as therapy control require methods with adequate sensitivity. Only few analytical techniques show sensitivity in the nano- and picogram range, one of those being gas chromatography with electon capture detection.

The electron capture detector exhibits a high response for compounds containing nitro and halogen groups as well as conjugated carbonyl groups. Only few drugs can be determined without conversion to a derivative with electrophoric properties. Among drugs that have been determined without derivatization, diazepam (1) and chlorpromazine (2) can be mentioned.

Many drugs, however, contain functional groups that can easily be acylated or alkylated. Acylations with halogen containing reagents have been performed with primary and secondary amines (3-8), alcohols (8-9) and phenols (10-12). The acylation of amides has also been reported (13). Drugs of the sulphonamide type can be determined after methylation and heptafluorobutyrylation (14).

Carboxylic acids can be alkylated to 2, 2, 2-trichloroethyl esters (15) and pentafluorobenzyl esters (16, 17). The alkylation of phenols by pentafluorobenzyl bromide has also been reported (17, 18).

On the other hand, there are a great many drugs that do not have any derivatizable functional group and other approaches are necessary.

Ring acylation of phenothiazine derivatives in the presence of a Friedel-Crafts catalyst has been reported by Noonan et al. (19), who also brominated the ring-system in some phenothiazines (20). Derivatives sensitive to electron

capture may be formed by oxidation. Drugs containing the diphenylmethane moiety can be oxidized to benzophenone with alkaline permanganate (21) or chromic acid (22, 23). Drugs of the benzodiazepine class can be hydrolyzed to form substituted benzophenones, which are determined by gas chromatography with electron capture detection (24, 25).

Scope of the Present Study

The majority of drugs are tertiary amines or quaternary ammonium compounds, and the main object of this study is to prepare derivatives suitable for electron capture detection of such compounds. Oxidation and dealkylation procedures have been extensively investigated. With the developed methods many drugs are now accessible for sensitive determinations also in biological samples.

Oxidation of gem-diphenyl substituted compounds to benzophenone by chromic acid is discussed in paper I, and in paper II the chromic acid oxidation product of pipradrol is presented. Alkaline permanganate is introduced as an alternative reagent for the oxidation in paper III.

Demethylation of tertiary amines is discussed in papers IV-VI. The transformation of the tertiary amine to a carbamate by reaction with chloroformate is presented in papers IV and V. The carbamate is hydrolyzed by acid to the corresponding secondary amine. This is perfluoroacylated to a product which is determined with good accuracy in an electron capture detector. The determination of a tertiary amine after transformation to an electrophore containing carbamate is presented in paper VI.

Oxidation and demethylation are examples of degradative derivative formation, and compounds in the sample containing the same reactive groups can be co-determined. Some metabolites of gem-diphenyl substituted drugs also give benzophenone on oxidation. Such an interference was suspected in the oxidation method of terodiline (22) and an alternative and selective method had to be developed and is presented in paper VII.

The present study also includes two papers, VIII-IX, dealing with the optimum conditions required for electron capture detection of benzophenones. Selective determinations of different benzophenones are possible since they react in the detector with different mechanisms.

Use of Derivatives in Electron Capture Gas Chromatography

The derivatization of organic compounds before electron capture gas chromatography is necessary mainly for two reasons:

1. Polar groups in the molecule must be covered. Compounds containing hydroxyl and amino groups are strongly adsorbed to the support in the gas chromatographic column, which makes quantitative determinations in the nanogram range impossible.

2. The sensitivity of the compound in the detector must be increased selectively by the introduction of electrophoric groups.

On the other hand, a practically useful derivatization must fulfill several requirements:

1. The derivative must have high chemical stability.

2. The reaction must be quantitative and has to proceed in a fairly short time.

3. Preferably only one reaction product should be formed. If several derivatives are formed and a reproducible ratio between the products is obtained, the method can be advantageously used in quantitative determinations as has been shown by Vessman et al. (22, 23, 25).

4. The purification of the reaction mixture must be simple. The lack of methods for easy removal of the excess of reagents make many potential electron capture reagents unusable for quantitative work.

Oxidation of Drugs to Conjugated Carbonyl Compounds

Oxidation of drugs is a well known method to increase the sensitivity of photometric determinations. Many drugs are oxidized with different reagents to carbonyl containing compounds (26-30). Alkaline permanganate oxidation of diphenylhydantoin to benzophenone has been reported by several authors (31-35). Photometric determinations after oxidation have also been performed for other gem-diphenyl containing drugs such as doxapram (36) and methadone (37).

The sensitivity increase is very high if compounds containing the gem-diphenyl moiety are converted to the corresponding benzophenone before electron capture gas chromatography. For example, meclastine (I. compound 37) can be determined with electron capture gas chromatography down to an injected amount of 2.5 ng, while after oxidation the formed chlorobenzophenone can be detected down to 10 pg (38).

Quantitative methods for the determination of the two drugs emepronium (23) and terodiline (22) are based on electron capture gas chromatography of the chromic acid oxidation product, benzophenone.

Meclastine can be determined after chromic acid oxidation and electron capture detection in serum samples after a peroral administration of one milligram (39). Brompheniramine is determined after alkaline permanganate oxidation (21).

Conditions for Chromic Acid Oxidation

The composition of the oxidation reagent, chromic acid in 6% sulphuric acid in glacial acetic acid, was worked out for the quaternary ammonium compound emepronium (23). It was shown that nonaqueous conditions must be used if benzophenone is to be formed (I). The chromic acid content of the reagent could be varied between 0.02 and 0.04 moles/l without influencing the yield, but the reaction rate was considerably affected (40). The oxidation of emepronium at room temperature was complete in 15 - 25 minutes (I) and a study with ^{14}C-terodiline (N-tert-butyl-1-methyl-3,3-diphenyl-propylamine) revealed no

unreacted material 25 minutes after the start of reaction (41). A study using ^{14}C-terodiline revealed acidic oxidation products besides benzophenone.

Yields of Benzophenone by Oxidation of gem-Diphenyl Substituted Compounds with Chromic Acid.

Further studies gave important information on the relation between structure and the benzophenone yield in the oxidation of diphenylmethane derivatives. It was also essential to clarify which compounds would interfere in the determination, and to find suitable internal standards in quantitative determinations. The precision in the oxidation yields was very good (± 1.6%. I. Table 7).

Diphenylmethane gave 87.5% of benzophenone, while benzhydrol was almost quantitatively transformed (I. Table 3). On introduction of an alkylamino chain at the α-carbon in benzhydrol the yield was reduced.

Benzhydryl ethers with an amino group in the aliphatic chain gave almost quantitative yields of benzophenone (93-99%, I. Table 7).

gem-Diphenylalkylamines gave in most cases yields varying between 50 and 80% (I. Table 4). However, compounds with an amino group directly attached to the α-carbon gave only 12% of benzophenone (I. Table 8). This low yield was found to be due to a low oxidation rate (40).

Some drugs, e.g. those without a hydrogen at the α-carbon or those having a carbonyl containing substituent on this carbon, did not form any benzophenone on chromic acid oxidation (I. Table 9).

Oxidation of Pipradrol by Chromic Acid

The chromic acid oxidation of pipradrol gave another product (I), which was identified as 6-benzoyl-1,2,3,4-tetrahydropyridine by spectroscopic means (II). The oxidation product has good sensitivity in an electron capture detector and a quantitative determination of pipradrol can be based on this product (II).

Oxidation by Alkaline Permanganate

Some of the drugs, which were not amenable to chromic acid oxidation, could be oxidized with alkaline permanganate. Permanganate was found to need extremely alkaline conditions and in most cases 2.5 M potassium hydroxide had to be used to get an adequate yield of benzophenone (III). Benzophenones are degraded in aqueous alkaline solutions, but compounds which are ionized in alkaline solution, e.g. acids and quaternary ammonium compounds, could be oxidized in a two-phase system. The formed benzophenone was in this system continuously removed to an organic phase (III). Most drugs containing the diphenylmethane moiety are amines, thus requiring a single-phase procedure (III). The precision in the two-phase oxidation was found to be superior to the single-phase one ($\pm$ 1.4% and $\pm$ 5% respectively, III).

Alkaline permanganate oxidation is of great value in the determination of drugs that can be oxidized in a two-phase system. The degradation of the formed benzophenone in a single-phase oxidation system can only be overcome by a very careful choice of internal standard.

Comparison of Oxidizing Agents

The yields of benzophenone by permanganate oxidation were in most cases comparable to those obtained by chromic acid oxidation. The main differences compared to chromic oxidation were:

1. Esters and amides gave yields of $>$95%. Chromic acid gives no benzophenone from such compounds.
2. Quaternary ammonium compounds gave much lower yields than the corresponding tertiary amines.
3. Compounds without a hydrogen at the α-carbon gave high yields of benzophenone, e.g. methadon (74%).

The degradation of the oxidation product, benzophenone, in alkaline permanganate is a disadvantage, without correspondence in chromic acid even at long reaction times. Chromic acid oxidation is preferred for this reason, when the two reagents give similar yields of benzophenone.

A third oxidation reagent, barium peroxide in sulphuric acid, has made it possible to determine methadone in a two-phase system with good precision by electron capture gas chromatography (42).

The detection limit in chromic acid oxidation is ~1 ng/ml due to a benzophenone blank (22, 23). The permanganate method gives a benzophenone blank of the same magnitude.

Advantages of Derivatization by Oxidation

Besides the sensitivity increase, there are other certain advantages of oxidative derivatization.

Most drugs with the diphenylmethane moiety are tertiary amines or quaternary ammonium compounds. These drugs cannot easily be converted to derivatives (except after demethylation) with good sensitivity in an electron capture detector. Furthermore, the polar amino groups are removed and disturbances by adsorption in the gas chromatographic column are avoided.

There is a further great advantage. Impurities are often completely decomposed in oxidation methods and the obtained chromatograms are very clean, which increases the precision of the method particularly in nanogram analyses. In acylations, for example, extraneous material are often derivatized and give rise to unwanted disturbances (VII, Fig. 5).

A disadvantage in the oxidation methods is the risk that metabolites will interfere in the determination. This will be discussed below.

Dealkylation

Losses of amines due to adsorption to the support in a gas chromatographic column is a serious analytical problem. A method to avoid this, applied to drugs with the amitriptyline structure, was methylation followed by a Hofmann degradation

to the corresponding olefin (43-44). However, derivatives of this kind have low electron capture sensitivity. A method which permits the determination of tertiary amines by electron capture gas chromatography is presented in paper IV. The tertiary amine is treated with ethyl chloroformate to form a carbamate, which is hydrolyzed by concentrated hydrobromic acid to the corresponding secondary amine. The secondary amine is heptafluorobutyrylated, with trimethylamine catalysis according to Walle and Ehrsson (7). Some tertiary amines, however, reacted in a different manner. Thus, on treatment with chloroformate methadone was deaminated (V), presumably to 2-ethylidene-5-methyl-3,3-diphenyltetrahydrofuran (45). A similar reaction has been reported for N,N-dimethyldibenzo(b.f.)thiepin-10-methylamine which is transformed to the corresponding chloromethylene derivative (46).

A carbamate lacks basic properties, thus making determinations of nanogram amounts possible by gas chromatography and mass fragmentography. Carbamates with electrophoric properties can also be determined by electron capture gas chromatography, as shown in paper VI.

Carbamate Formation

The conversion of Recipavrin^{R} (compound 1, IV) to the corresponding carbamate required a great excess of ethyl chloroformate and heating on a water bath. A study of several alkyl chloroformates revealed that methyl and propyl chloroformates are preferable as they react more rapidly (V). Benzyl chloroformate reacts very slowly, but when a basic catalyst, sodium carbonate, was introduced, a complete carbamate formation was obtained in less than 30 minutes from Recipavrin^{R} (V). The positive effect of base catalysis has also been demonstrated for other alkyl chloroformates (V).

Carbamates are very stable, except for the derivatives from bromoethyl chloroformate which are probably decomposed by bromine solvolysis (VI). The reaction between chloroformate and Recipavrin^{R} gave two side products (IV). The side products are formed with all chloroformates and no difference in the yield ratio of the products has been observed (V).

Hydrolysis of Carbamates

Hydrolysis of the ethyl carbamate of Recipavrin[R] could be performed in concentrated hydrobromic acid in two hours (IV). In more dilute acid the reaction rate decreased. The most easily hydrolyzed carbamate was the one from the methyl derivative. All methyl carbamates were hydrolyzed with the same rate (V). The treatment with concentrated acid often resulted in an attack on other functional groups in the molecules (ester, amide, ether etc.). Another reagent, hydrobromic acid in acetic acid 30%, could hydrolyze methyl carbamates in less than 10 minutes (V). This reagent was also milder to other groups in the molecules (V). The carbamates were resistent to alkali (V).

Applications of the Chloroformate Method

The method described in paper IV gave an overall yield of 75% of the heptafluorobutyrylated demethylation product of Recipavrin[R]. The major source of losses is probably the formation of the two side products in the chloroformate reaction. By use of an internal standard, which behaved in a similar way to Recipavrin[R] it was possible to perform quantitative determinations in the ng range (IV). A modified reaction route (V) enabled the determination of other amines, e. g. tertiary amines among drugs used as antidepressants. For example amitriptyline gave an absolute yield of 78% of heptafluorobutyrylated nortriptyline, while imipramine gave 66% of heptafluorobutyrylated desipramine. The determination of imipramine by gas chromatography can be performed according to the chloroformate method with chloroimipramine as internal standard (47).

The weak step in the procedure given in IV and V is the hydrolysis. The methylcarbamate of diphenylhydramine gave benzhydrol on hydrolysis (V).

Electrophoric Chloroformates

A simplification of the method could be obtained by the use of electrophoric chloroformates; pentafluorobenzyl carbamate was suitable for this purpose. The reaction could be promoted by base catalysis and was completed in 30 minutes. Three other carbamates containing electrophoric groups were also tested but the pentafluorobenzyl carbamate was found to be superior with respect to stability

and sensitivity. As little as 3 pg of pentafluorobenzyl carbamate can be detected in an injected sample (VI). The new reagent has been applied in quantitative determinations, in the µg/ml range, with good precision (VI).

Choice of Internal Standard

Although neither oxidation nor demethylation give a quantitative yield of electron capturing derivatives, the methods can be used in quantitative determinations. With a proper internal standard, both good precision and a relative recovery of 100% is obtained (IV, VI, 22, 23, 25, 47).

The functions and the properties of a good internal standard in electron capture gas chromatography are summarized below:

a) If it is added to the sample, it can compensate for transfer losses. This means compensation for incomplete phase separations as well as for adsorption losses to the glass walls in the reaction vessels.

b) It must have about the same distribution coefficient as the object.

c) It must react in the same manner as the object in the derivatization step. The configuration around the reacting group has to be very similar in both compounds (Cf. terodiline VII).

d) It must be well separated in the chromatograms just behind the object.

e) It must react with the same mechanism as the object in an electron capture detector.

Selectivity of the Derivatization Methods

The determination of a compound from biological material often comprises four steps; extraction to an organic solvent, purification of the extract, derivatization and gas chromatographic determination. In the extraction procedure, good selectivity can be obtained by the use of a moderately solvating organic phase, if necessary combined with a complexation, e.g. ion pair technique, c.f. Berlin et al. (48) and Modin and Bäck (49).

In degradative derivatization there is always a risk that metabolites will be determined together with the unchanged drug. Side chain metabolites will interfere in the oxidation of drugs having the diphenylmethane moiety. If such an interference is suspected a more selective method must be used. An example of this kind with the secondary amine terodiline is presented in paper VII. Terodiline is heptafluorobutyrylated and determined by electron capture gas chromatography. The benzophenone method (22) and the heptafluorobutyrylation (VII) methods are equal in sensitivity. The determination of serum levels of terodiline with the two methods indicates that only minor amounts of metabolites can be co-determined in the benzophenone method since the levels were found to be almost equal (VII).

In the demethylation procedures there is also a possible source of interference. One common route of metabolism of tertiary amines is demethylation to secondary amine. Secondary and tertiary amines form with chloroformate the same carbamates (V). Thus secondary amines must be excluded, for example by acylation, before the chloroformate reaction.

Recovery and Precision

If the internal standard is chosen according to the principles outlined above, the relative yield in procedures which include derivatizations is 99 - 100% with a precision better than ± 5% (IV, VI, VII). The absolute recovery of the object can be determined with radioactive material, and it is then possible to control losses in all extraction and derivatization steps. Such an experiment was done with heptafluorobutyrylated terodiline (VII). Losses were only observed in the back extraction of terodiline to an acidic aqueous phase. Those studies also indicated that the needed time of extraction increased with higher serum concentration.

Electron Capture Studies

Electron Capture Mechanisms

Several studies have been made on the mechanism of electron capture (5, 50-53). Wentworth et al. (54-56) have studied such phenomena from a theoretical point of view and suggested three main mechanisms for electron capture.

The mechanism can be non-dissociative or dissociative. The non-dissociative, or better the resonance mechanism, can be illustrated as follows:

$$AB + e^- \rightleftharpoons AB^- \qquad \text{I}$$

The capture of an electron in this mechanism is favoured by low detector temperature. Substances reacting according to this mechanism are polyaromatic hydrocarbons and aromatic carbonyl compounds.

The dissociative mechanisms can be illustrated by the formulas:

$$AB + e^- \longrightarrow A^{\cdot} + B^- \qquad \text{II}$$

$$AB + e^- \rightleftharpoons AB^- \longrightarrow A^{\cdot} + B^- \qquad \text{III}$$

Aliphatic halogenides react according to mechanism II, while mechanism III is found among aromatic halogenated compounds. The dissociative mechanisms, II and III, are either favoured by high temperature or are insensitive to temperature changes.

The above mechanisms have later been confirmed by Karasek with the aid of plasma chromatography (57-59).

Temperature Dependence in Electron Capture Detectors working in the Pulsed Mode

The electron capture detectors can operate according to two different principles, pulsed and D. C. mode, both of which have their advantages from an analytical chemical point of view. The pulsed mode detector collects the electrons during short intervals, while in the D. C. mode detector the electrons are removed continuously.

Wentworth and Chen (54) have pointed out the importance that electron capture mechanism studies are performed in the pulsed mode detector type, where the energy of the electrons is slightly above thermal.

A study of the temperature dependence of substituted benzophenones in a detector working in the pulsed mode is presented in (IX). For convenience, the results were presented in plots according to Wentworth, which give the response as the conditional electron capture coefficient, K′ versus temperature (VIII). The plots presented in (IX) have about the same shape as those reported for acetophenones by Wentworth and co-workers. (56, 60). This indicates that benzophenone and its derivatives with electron donating substituents (alkyl, methoxy) as well as fluorobenzophenones reacted according to the resonance mechanism, I (IX). The highest response is obtained at 120°C. Increase of temperature gives a considerable decrease in sensitivity. The response of benzophenone at 225°C was only 3% of that at 120°C (IX).

p-Chlorobenzophenone shows a typical mechanism III behaviour, as reported by Steelhammer for p-chloroacetophenone (60). Benzophenones with bromo, iodo or nitro substituents gave temperature independent response in the pulsed mode detector.

In a recent investigation (61) it has been possible to study the temperature dependence of benzophenone and its p-methyl and p-chloro analogues in a pulsed detector with frequency modulation. A temperature change from 100° to 350° decreased

the sensitivity by a factor of 2×10^6 for benzophenone and 3×10^6 for the p-methyl analogue respectively, but only 3 times for p-chlorobenzophenone.

From the above it is obvious that a proper control of the detector temperature is of utmost importance. It is also apparent that the detection of a compound by mechanism III can be promoted by a temperature increase, as the response of an unwanted compound reacting according to mechanism I will be successfully suppressed at high detector foil temperature in a pulsed mode detector.

Temperature Dependence in a D. C. Mode Detector

Studies with benzophenones in three different types of D. C. mode detectors showed that all compounds have a temperature independent region at low detector foil temperature. Benzophenones with electron donating substituents had a much later onset of temperature-dependence than in the pulsed detector (VIII, IX).

In electron capture detectors working with a constant current between the electrodes (D. C. mode), the electrons have an energy which is above the thermal level. The higher energy of the electrons in the D. C. mode detector was apparent from the temperature dependence studies of acetophenone and chloro-acetophenone (VIII, Fig. 3). A sharp negative slope of acetophenone at higher temperatures was obtained and has also been shown by Durbin et al. in a pulsed mode detector (62). The temperature dependence of the benzophenones in the D. C. mode detectors shows the same plots as in the pulsed detector but the slopes had a later onset. The results show that Wentworth's theoretical models are also valid in a detector working with electrons with an energy above the thermal level.

Comparison of Pulsed and D. C. Mode Detectors

From the study of the temperature dependence of benzophenones, it is apparent that the sensitivity is about equal in all detectors (IX, Table 2), and that the sensitivity order between the different benzophenone derivatives is the same except for p-iodo benzophenone.

The sensitivity ratio between the ^{3}H and ^{63}Ni detector (Varian 1400, optimal temperature) is only about 3 and much less than that reported by Matin and Rowland (5) and Wilkinson (63).

The pulsed mode detector has particular advantages for the detection of a compound reacting according to mechanism III in a mixture with extraneous compounds belonging to mechanism I (e. g. dibutyl phthalate, IX), as discussed above. On the other hand, in some cases the detection of mechanism I compounds must take place at a detector foil temperature far below the optimum sensitivity due to a too high column temperature. Furthermore, small deviations in the detector temperature will cause great sensitivity changes for compounds belonging to mechanism I, thus giving uncertain results.

A D. C. mode detector does not suffer from the last two drawbacks since all compounds show a temperature independent region at low detector temperature. This detector must be the one of choice in determinations of benzophenone and similar conjugated carbonyl compounds.

Sensitivity Studies in Electron Capture Detectors

When a sensitivity value is given, it is obvious that the actual detector foil temperature must be stated as well as the type of detector used. The sensitivity values are usually expressed in moles/second. These values are independent of retention times and separating efficiency of the column.

Electron Capture Response of Conjugated Carbonyl Compounds

The electron capture response of a compound depends on its ability to capture an electron and to stabilize the captured electron in the molecule (5). The high electron capture response of conjugated carbonyl compounds is probably due to their ability to stabilize a captured electron. This stabilization theory can be supported by the fact that a good correlation exists between the Hammet σ-values and the sensitivity for benzophenones with electron withdrawing substituents at a foil temperature of 170° (VIII, Fig. 8).

The minimum detectable quantity of benzophenone is 3×10^{-15} moles/second, which corresponds to an injected amount of about 10 picograms (VIII). This means that benzophenones have a sensitivity comparable to that of heptafluorobutyryl and 2,4-dinitrophenyl derivatives (VIII, Table 6).

Electron Capture Response of Perfluoroacyl Derivatives

Heptafluorobutyrylated amines show excellent sensitivity in an electron capture detector and they are independent of temperature changes in the detector (IV, VII). This is in line with the findings of Pettitt et al.(64).

The sensitivity limit of the methods for determination of the drugs in serum is lower due to the presence of interfering components in the chromatograms (IV, VII).

Increase of Response by Intramolecular Hydrogen Bonding

The oxidation product of pipradrol shows an extraordinary high response compared to similar carbonyl compounds (II, Table 1). After comparison with two reference compounds, viz. 2-benzoyl-piperidine and N-methyl-2-benzoyl-piperidine, it is apparent that the increase in the electron capture response is due to an intramolecular hydrogen bond (II). An intramolecular hydrogen bond is possible in 6-benzoyl-1,2,3,4-tetrahydropyridine, and the response is comparable to that of benzophenone (II).

Electron Capture Response of Electrophoric Carbamates

One alkylhalogenated and three benzyl chloroformates were studied as reagents for direct determination of tertiary amines by electron capture gas chromatography (VI). As expected, the pentafluorobenzyl derivative showed the highest sensitivity and suitability for quantitative determinations. High sensitivity values have also been reported for pentafluorobenzyl ethers (18). The response of the derivative was independent of detector temperature in a D. C. mode detector.

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